

## **Clinical significance of CYP2C9-status guided valproic acid therapy in children**

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## Summary

**Objectives:** Valproic acid (VPA) induced adverse effects, which are sometimes serious in children, can be associated with alterations in VPA metabolism. VPA-evoked toxicity is attributed to both the parent compound and its unsaturated metabolites, primarily formed by CYP2C9 enzyme. Thus, patients' CYP2C9-status may account for the predisposition to adverse reactions, and testing CYP2C9-status may contribute to the improvement and rationalization of VPA therapy in children.

**Methods:** In CYPtest group, children's CYP2C9-status was screened before initiating antiepileptic therapy. CYP2C9-status was estimated by the identification of defective *CYP2C9* allelic variants (*CYP2C9\*2*, *CYP2C9\*3*) and current CYP2C9 expression in patients' leukocytes which reflects hepatic CYP2C9 activities. Combining the results of CYP2C9 genotyping and CYP2C9 expression, the patients' VPA-metabolizing capacity was predicted, and VPA dosing was adjusted to the patients' CYP2C9-status. Clinical and biochemical parameters, such as VPA serum levels, blood cell counts, liver function parameters, adverse effects in patients of CYPtest group were compared with those of the control group treated with VPA according to conventional clinical practice.

**Results:** CYP2C9-guided treatment significantly reduced VPA-misdosing and consequently decreased the ratio of patients out of the range of target VPA blood concentrations. In CYPtest group of children receiving CYP2C9-status adapted dose, serum alkaline phosphatase (ALP) and the ratio of patients with abnormal ALP levels were substantially lower than in the control group. The incidence of serious side effects, notably hyperammonemia, was reduced in CYPtest group; however, some other side effects, such as weight changes and somnolence, could not be avoided.

**Significance:** The knowledge of pediatric patients' CYP2C9-status can contribute to the optimization of VPA dosing and to the avoidance of misdosing-induced side effects.

**Key words:** anticonvulsant therapy, personalized pharmacotherapy, cytochrome P450, CYP2C9 genotype, CYP2C9 expression

## Introduction

Since its introduction as an antiepileptic drug, valproate (VPA) became one of the first choices in treatment of generalized or partial seizures. VPA is well-tolerated; however, serious complications, including hepatotoxicity, hyperammonemic encephalopathy, bone marrow suppression, and bone metabolic disorders, rarely occur in patients.<sup>1</sup> Infants are particularly vulnerable to VPA-injury, although the pathogenesis is still not clear.<sup>2,3</sup> Genetic and non-genetic factors may increase the predisposition to VPA-induced adverse reactions; thus, recognition of risk factors can contribute to the avoidance of adverse events.

In adult patients on VPA monotherapy, the majority of the dose is eliminated as glucuronide-conjugates. Mitochondrial  $\beta$ -oxidation, producing unsaturated VPA-metabolites, is the second major metabolic pathway. About 15-20% of VPA-dose is metabolized by cytochrome P450 (CYP) enzymes, resulting in the formation of 4-ene-VPA and hydroxy-metabolites.<sup>4</sup> The main catalyst of hydroxylation and desaturation to 4-ene-VPA is CYP2C9 with minor contribution of CYP2A6 and CYP2B6.<sup>5,6</sup> Although the mechanism of VPA-induced adverse effects is not clearly understood, the parent compound and its unsaturated metabolites are postulated to be associated with VPA toxicity.<sup>7</sup> Despite the fact that CYP-mediated oxidation contributes to a minor part of VPA-metabolism in adults, in children the CYP-catalyzed pathways become the principal route of metabolism. It is supported by the facts that i) hepatic glucuronidation activities are low in infants, primarily in children younger than two years of age, and UDP-glucuronyl transferases involved in VPA-conjugation are expressed under adult levels until sometimes after 10-15 years of age;<sup>8,9</sup> ii) VPA and some VPA-metabolites inhibit mitochondrial  $\beta$ -oxidation;<sup>10,11</sup> iii) CYP-dependent metabolism in children exceeds adult activities and decreases to adult levels by puberty.<sup>12,13</sup> As a result, larger proportion of VPA is liable to CYP-dependent metabolism in children than in adults, which explains the increase in VPA clearance and toxic metabolite formation in pediatric patients. At

therapeutic doses, VPA elimination half-life is substantially shorter (6-9 hours) in children than in adults (10-20 hours).<sup>14</sup> Furthermore, shifting the metabolic pathways may lead to age-related differences in the incidence of adverse reactions.

Several *CYP2C9* allelic variants, resulting in the synthesis of non-functional *CYP2C9* enzyme, have been identified with the highest frequency of *CYP2C9*\*2 and *CYP2C9*\*3 in Caucasian population.<sup>15</sup> Patients with mutated genes are permanent poor metabolizers, whereas those with wild-type alleles (*CYP2C9*\*1/\*1) have the potential for the expression of functional enzyme. However, environmental (nutrition, medication) or internal factors (age, diseases) can transiently modulate *CYP2C9* expression, leading to altered *CYP2C9* activities and transient poor (or extensive) metabolism, even in the patients with *CYP2C9*\*1/\*1 genotype. As a consequence of genetic and non-genetic variations, a patient's drug-metabolizing capacity can be weaker (or more extensive) compared with the other members of the population.<sup>16</sup> Patients' *CYP2C9*-status can be estimated by the identification of defective *CYP2C9* alleles and by current *CYP2C9* expression. We have previously described that *CYP2C9* expression in leukocytes of those subjects who do not carry loss-of-function mutations reflects the hepatic tolbutamide hydroxylation activity selective for *CYP2C9*.<sup>17</sup> *CYP2C9*-genotyping for *CYP2C9*\*2 and *CYP2C9*\*3 identifies the genetically determined poor metabolism, whereas *CYP2C9* expression in leukocytes of patients with wild-type alleles estimates reduced or even increased *CYP2C9* activity resulted in by non-genetic variations. We have reported in a retrospective study that children's *CYP2C9*-status influences the serum VPA concentrations and consequently the dose-requirements.<sup>18</sup> For optimal therapeutic levels, the VPA dose-requirement was substantially higher (33 mg/kg) in normal expressers carrying *CYP2C9*\*1/\*1 genotype than in low expressers or in patients carrying any polymorphic *CYP2C9* alleles (14–17 mg/kg). Our present work aimed to prospectively investigate whether preliminary assaying of *CYP2C9*-status and *CYP2C9*-status guided VPA therapy have potential clinical benefit for pediatric patients, and whether personalized drug therapy can reduce the risk of VPA-

misdosing induced adverse reactions.

## **Methods**

### **Patients and sampling procedure**

Pediatric patients (N=99) suffering from epilepsy diagnosed with partial or generalized seizures were enrolled in the study carried out at Heim Pál Children's Hospital and at the 2<sup>nd</sup> Department of Pediatrics, Semmelweis University (Budapest, Hungary). We recruited patients, younger than 15 years of age, who were newly-diagnosed with epilepsy and were to be applied VPA therapy. The patients were excluded if their VPA therapy was interrupted. The patients' clinical data were collected between 2006 and 2014. From 2010, each child was CYP2C9-tested at the beginning of antiepileptic therapy. CYPtesting of patients was approved by the Hungarian Committee of Science and Research Ethics. The representatives of each patient gave their informed consent to participate in the study. The patients' demographic data (Table 1) and the details of anticonvulsant therapy were recorded. The patients were not given any other medication, but VPA as mono-therapy (Convulex or Depakine). The hematological (red and white blood cell and platelet counts) and biochemical parameters (serum alkaline phosphatase, aspartate transaminase, alanine transaminase,  $\gamma$ -glutamyl transferase, calcium, phosphorus) were checked at the beginning of the anticonvulsant therapy and monitored regularly. Blood ammonia level was not routinely assayed, only if any symptom reasoned it. All signs of adverse reactions due to the VPA treatment were reported, and were classified as mild (somnolence, fatigue, enuresis, hair loss) or severe (hyperammonemia, hematological disorders, confusion) side effects.

### **CYP2C9-testing**

In CYPtest group, the patients' CYP2C9-status was determined by CYP2C9 genotyping and by assaying CYP2C9 expression in leukocytes before the beginning of anticonvulsant therapy. Genomic DNA and leukocytes were isolated from the samples of

peripheral blood according to the methods described by Temesvári et al.<sup>17</sup> CYP2C9 genotyping was carried out by hydrolysis single-nucleotide-polymorphism analysis for *CYP2C9\*2* and *CYP2C9\*3* using TaqMan Probes (BioSearch Technologies, Novato CA). For assaying CYP2C9 expression, total RNA was isolated from leukocytes, RNA was reverse-transcribed into single-stranded cDNA, and real-time PCR with human cDNA was performed using UPL probe for CYP2C9 (Roche Diagnostics, Mannheim, Germany). The quantity of CYP2C9 mRNA relative to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase was determined. Three categories of CYP2C9 expression were distinguished to describe low, normal and high expressers. The cutoff values for the CYP2C9 mRNA levels in leukocytes were previously established on the basis of the cutoff values for the hepatic CYP2C9 activity (tolbutamide hydroxylation), allowing a distinction between low, normal (medium) and high expressers ( $5 \times 10^{-6}$  and  $2.5 \times 10^{-5}$ , respectively).<sup>17</sup>

### **VPA dosing**

In CYPtest group (N=52), the patients' anticonvulsant therapy was guided by their CYP2C9-status: i) non-VPA therapy was proposed to the children with two mutated *CYP2C9* alleles, ii) and VPA therapy adjusted to the patients' CYP2C9-status (CYP2C9 genotype and CYP2C9 expression) was applied in those with one or two wild-type alleles.<sup>18</sup> Normal dose (30-40 mg/kg) was given to the normal CYP2C9 expresser patients carrying CYP2C9 homozygous wild genotype (*CYP2C9\*1/\*1*), reduced dose (10-20 mg/kg) was administered to the children with heterozygous genotypes (*CYP2C9\*1/\*2* or *CYP2C9\*1/\*3*), or to low CYP2C9 expressers, while increased dose (>40 mg/kg) was targeted in high expresser patients with *CYP2C9\*1/\*1* genotype. In control group (N=47), the target dose of VPA was adjusted to the patients' body weight (20-40 mg/kg) according to the standard clinical protocol, and was modified if the signs of adverse reactions or non-responsiveness required.<sup>19</sup>

### **Serum VPA assay**

The blood samples for drug assays were taken from the patients before the morning dose and VPA concentrations were assayed every second week after the beginning of VPA treatment. The steady-state serum concentration of VPA was determined by fluorescence polarization immunoassay method (AxSYM Valproic Acid Assay, Abbott Laboratories, IL). The VPA concentrations between 40 and 100 µg/ml were considered to be in the reference range.<sup>19</sup> The two-week sample was used for checking VPA serum concentration, and dose was modified if exposure was outside the target range. The serum levels measured at the fourth week were considered to be the stable steady-state concentration, and the dose prescribed at this time was considered to be the maintenance dose.

### **Statistical analysis**

Statistical analysis of biochemical and hematological parameters as well as VPA serum levels was carried out using GraphPad Instat (v3.05, GraphPad Software, San Diego, CA). Parameter distributions were analyzed by Kolmogorov-Smirnov test. Between-group differences were calculated by Mann-Whitney U-test. The benefit of CYP2C9-status guided VPA therapy over the classical dosing was also evaluated comparing the ratio of patients with therapeutic VPA serum levels, normal hematological or biochemical parameters in CYPtest group with those in control group. The frequencies of adverse events were also compared. The differences between CYPtest and control groups were calculated by Fisher's exact test. A *P* value of <0.05 was considered statistically significant.



## Results

### Assaying CYP2C9-status of pediatric patients

Because of homozygous mutant genotype (*CYP2C9*\*2/\*2), one of the CYPtested children was excluded from the study, and non-VPA therapy was applied. All of the remaining patients in CYPtest group (N=51) expressed at least one wild-type *CYP2C9* allele (*CYP2C9*\*1), and more than one quarter of patients carried one of the polymorphic *CYP2C9* variants (*CYP2C9*\*2 or *CYP2C9*\*3) (Table 1). The *CYP2C9* genotype frequencies of *CYP2C9*\*1/\*2 and *CYP2C9*\*1/\*3 in the patients (13.5% and 11.5%, respectively) were similar to those in Caucasian populations.<sup>15</sup> *CYP2C9* expression assay displayed low expressers in two thirds of patients with homozygous wild genotype (*CYP2C9*\*1/\*1) (Table 1). Subjects with two wild-type alleles are generally considered to be extensive metabolizers; however, low *CYP2C9* expression was predicted to result in poor VPA metabolism in patients.<sup>18</sup> The unusually high ratio of low expressers was attributed to down-regulation of *CYP2C9* expression by cytokine release in epilepsy.<sup>18</sup> Nine children with heterozygous *CYP2C9* genotypes (*CYP2C9*\*1/\*2 or *CYP2C9*\*1/\*3) expressed *CYP2C9* mRNA at normal levels, although they were considered to be poor metabolizers because they carried merely one mutant allele, resulting in reduced *CYP2C9* function. Of 51 children in CYPtest group, altogether 38 patients (25 low expressers with *CYP2C9*\*1/\*1 genotype and 13 patients carrying heterozygous genotype) were expected to have poor VPA metabolizing capacity.

### Potential benefit of CYP2C9-status guided VPA therapy

For 74.5% of the patients in CYPtest group, who were low *CYP2C9* expressers or normal expressers with mutant *CYP2C9* alleles, reduced VPA dose (10-20 mg/kg) was targeted; whereas normal target dose (30-40 mg/kg) was administered to the normal expresser patients with *CYP2C9*\*1/\*1 genotype (23.5% of the patients) and increased dose (>40 mg/kg) was applied to the high expresser child carrying *CYP2C9*\*1/\*1 genotype. Despite the fact that

reduced VPA dose was administered to most of the children in CYPtest group, the therapeutic efficacy (seizure frequency) was similar to those in the control group of patients on conventional VPA therapy. Although the average VPA serum concentrations in CYPtest group did not differ significantly from those in control group (Table 2), the CYP2C9-status guided VPA dosing substantially reduced the number of patients displaying VPA serum levels out of the therapeutic range (9/51 vs 21/47) (Figure 1). Moreover, the deviation of VPA concentrations from the therapeutic range (40 to 100 µg/ml) was significantly lower in the CYPtest group than in the control group (Table 2).

For recognizing the early signs of adverse reactions, biochemical and hematological parameters were followed in both groups from the beginning of anticonvulsant therapy. Before the beginning of antiepileptic therapy, the liver function parameters, serum levels of calcium and phosphorus as well as red blood cell, white blood cell and platelet counts were in the reference ranges in all patients. One month after the beginning of VPA therapy, no significant alterations were observed in red or white blood cell and platelet counts. Biochemical parameters of patients in both groups were also in reference ranges, except for the alkaline phosphatase (Table 2). The patients in the control group displayed a significant increase in serum alkaline phosphatase activity which is generally associated with either hepatotoxicity or bone metabolic disorders. Nevertheless, the levels of neither serum transaminases and  $\gamma$ -glutamyl transferase nor calcium and phosphorus in control patients differed from those parameters in CYPtest group. Serum alkaline phosphatase levels show great variation with age in children<sup>20</sup>; however, the substantial increase within one month was rather attributed to VPA treatment than to the transient hyperphosphatasemia. The age of the control patients were considered to be similar to that of the children in CYPtest group, whereas serum alkaline phosphatase activities exceeded the normal range in almost half of the control patients and merely in 4% of CYPtested patients. Furthermore, VPA-associated serious adverse effects

were observed more frequently in the control group than in the CYPtest group (Table 3). Hyperammonemia ( $>50 \mu\text{mol/l}$ ), the most frequent side effect in control patients (17%), was always accompanied with elevated levels of alkaline phosphatase (higher than 750 unit/l or even more than 1200 unit/l) and with other adverse reactions, such as somnolence, fatigue, consciousness or behavior disturbances. Furthermore, serum VPA concentrations in the patients with increased ammonia levels were found to be higher than 100  $\mu\text{g/ml}$ . It should be mentioned that the prevalence of mild side effects, including weight gain, hair loss, enuresis and somnolence was similar in the control and CYPtest groups (Table 3).

## Discussion

VPA has therapeutic benefits with a favorable safety profile, being well-tolerated in most patients; however, considerable attention has been paid to age-related differences in the incidence of VPA-associated adverse events.<sup>7,9,12</sup> Metabolic differences between children and adults, resulting in various developmental pattern in biotransformation, may play role in different side effect profiles. Several authors described the changes in VPA metabolic profile from childhood to adolescence and adulthood.<sup>7,9,21,22</sup> Glucuronide formation as the major metabolic pathway of VPA has been reported to be much lower in children than in adolescents.<sup>23</sup> Altered  $\beta$ -oxidation of VPA in pediatric patients, resulting in distinct metabolite profiles, have also been associated with liver dysfunction.<sup>21,23</sup> Assigning a prominent role in VPA metabolism to CYP2C9 in children, genetic variations in *CYP2C9* as the potential risk factors have been considered to contribute to VPA-induced toxicity.<sup>5,6,24</sup> Ho et al. demonstrated that VPA metabolizing activity was substantially lower in hepatic microsomes expressing *CYP2C9*\*2 or *CYP2C9*\*3 than the wild-type allele.<sup>5</sup> Statistically significant increase in VPA plasma concentrations was displayed in Chinese patients carrying *CYP2C9*\*3 allele<sup>24</sup>; however, the moderate differences may be attributed to the fact that the authors did not

take the age-related variations in VPA metabolism into account. In contrast, Guo et al. did not find any obvious impact of *CYP2C9*\*3 mutation on VPA plasma concentrations and dose-requirements in Chinese pediatric patients<sup>25</sup>, most likely because the authors neglected *CYP2C9* expression in patients. Our previous work has clearly demonstrated that *CYP2C9*-status of children younger than 15 years of age was associated with VPA dose-requirements for the optimal serum VPA levels.<sup>18</sup> The loss-of-function mutations in *CYP2C9* gene (*CYP2C9*\*2 or *CYP2C9*\*3) resulting in poor metabolism can lead to high blood concentrations of certain drugs (e.g. VPA, warfarin, hypoglycaemic drugs), causing more severe and frequent side effects<sup>14,26-28</sup>; however, we consider low *CYP2C9* expression to be an additional risk factor for undesired side effects of VPA in children.<sup>18</sup> Increased sensitivity of children to VPA can be explained by the poor glucuronidation of VPA, shifting the metabolic pathways towards *CYP2C9*-mediated metabolism and by the genetic and non-genetic factors influencing *CYP2C9* activity; thus, personalized VPA therapy adjusted to the patients' *CYP2C9*-status is recommended.

Hematological disorders, hepatotoxicity and hyperammonemia are rare adverse effects induced by VPA exposure, but sometimes result in fatalities.<sup>1,2,7,21,23</sup> VPA is hypothesized to have direct toxicity on bone marrow, whereas VPA itself and some of its metabolites might be involved in liver injury.<sup>1,7,22</sup> According to the *CYP2C9*-status guided anticonvulsant therapeutic strategy, non-VPA treatment is proposed to children carrying *CYP2C9* alleles with two loss-of-function mutations (*CYP2C9*\*2/\*2, *CYP2C9*\*3/\*3 or *CYP2C9*\*2/\*3) to avoid adverse reactions, whereas refinement of VPA dosing is proposed for those carrying one or two wild-type *CYP2C9* alleles.<sup>18</sup> The conventional dosing approach, targeting VPA daily dose of 20 to 40 mg/kg, has been found to be appropriate merely for normal *CYP2C9* expressers with *CYP2C9*\*1/\*1 genotype, whereas low expressers or children with one wild-type *CYP2C9* allele have been demonstrated to require substantially reduced dose.<sup>18</sup> *CYP2C9*\*1/\*1 genotype, predicted to be translated to an extensive metabolizer phenotype, can be switched into poor

metabolism due to phenoconversion, which eventually influences the manifestation of VPA side effects.<sup>16</sup> Furthermore, the genotype-phenotype mismatch results in more poor metabolizers than it would be predicted from CYP2C9 genotype. Several external or internal factors, including co-medications, nutrition, disease or hormonal changes, modifying the expression and activity of CYP2C9 enzyme, can give rise to phenoconversion of patients' *CYP2C9\*1/\*1* genotype.<sup>16</sup> Nevertheless, the variability of CYP2C9 expression in the children can hardly be explained by co-medications, because the patients on multi-drug therapy were excluded from the study. The down-regulation of CYP2C9 expression by pro-inflammatory cytokines released in patients following seizures is a logical explanation for poor metabolism.<sup>29</sup> Due to the wide range of the children's age, various hormonal statuses could also be the factor resulting in the variability of CYP2C9 expression.<sup>30</sup> However, we could detect all these influences by measuring CYP2C9 mRNA levels in the patients of CYPtest group and adjusted VPA dosing to their CYP2C9 expression. The conventional VPA-based antiepileptic therapy would have been inappropriate for most of the CYP2C9-tested children in the present study: one child carrying *CYP2C9\*2/\*2* would have poorly tolerated VPA, and 75% of the patients in CYPtest group would have been misdosed. Since the routine protocol of the conventional therapy orders checking VPA blood concentrations three to four weeks after the beginning of VPA therapy, there is no other guide, but symptoms that can inform about VPA-misdosing.<sup>19</sup> Therefore, much caution is recommended in clinical and biochemical monitoring of patients primarily during the dose escalation.

The benefit of CYP2C9-status guided VPA dosing over the symptom-driven therapy was clearly demonstrated by more precise VPA dosing and by successfully reduced risk of side effects. In CYPtest group, the knowledge of patients' CYP2C9-status before the beginning of anticonvulsant therapy guided the selection of the appropriate drug and the optimal dose. CYP2C9-status controlled VPA therapy improved the achievement of target VPA serum levels resulting in fewer patients out of the therapeutic range of serum VPA concentrations.

Moreover, alkaline phosphatase activity was significantly lower (in normal range) in the children on CYP2C9-status controlled therapy than in the patients on conventional symptom-driven therapy. Abnormalities in serum alkaline phosphatase are considered to be associated with bone metabolic disorders or hepatotoxicity. Decreased bone mineral density accompanied with reduced bone formation has been reported in children on long-term (more than six-month) VPA therapy.<sup>31</sup> The patients of the present study received VPA for only one month, and the serum concentrations of calcium and phosphorus were in normal range; thus, increased levels of serum alkaline phosphatase in control patients might be the marker of other pathomechanisms. Hyperammonemia, which is associated with impaired urea cycle in liver<sup>32</sup>, was frequently observed in the control children, and always accompanied with extremely elevated levels of alkaline phosphatase and increased serum concentrations of VPA (>100 mg/ml) in patients. An increase in blood ammonia level has poor predictive value for hepatotoxicity; thus, it was not routinely determined for recognizing hepatotoxicity. However, elevated blood ammonia level, often without an increase in liver function parameters, is associated with VPA-induced encephalopathy. The blood ammonia level is recommended to be measured if patients present any of the symptoms of nausea, vomiting, lack of appetite, fatigue, drowsiness, confusion, cognitive slowing or loss of consciousness.<sup>33</sup> At least one of these symptoms was observed in the hyperammonemic children in this study.

Our present work is the first attempt to establish the influence of the genetic and non-genetic variations of CYP2C9 on VPA metabolism. Our major findings indicated that personalized anticonvulsant therapy can be applied in pediatric patients by revealing the allelic variants in *CYP2C9* gene and the current CYP2C9 expression in patients' leukocytes. CYP2C9-status guided VPA treatment could prevent patients from misdosing and from exaggerated serum concentrations of VPA, resulting in the manifestation of toxic symptoms, such as increased alkaline phosphatase and hyperammonemia. Tailored VPA treatment

adjusted to the patients' CYP2C9-status has been demonstrated to be able to improve the safety of anticonvulsant therapy in one of the most vulnerable patient populations.

### **Key points**

- In pediatric patients, the metabolic pathways of valproic acid seem to be shifted towards the CYP2C9-catalyzed oxidation.
- Inferring the patients' valproate metabolizing phenotype from CYP2C9 genotype and CYP2C9 expression can lead to appropriate prediction.
- The children's CYP2C9-status influenced the serum valproate concentrations and consequently the dose-requirements.
- Patients' CYP2C9-status accounts for the predisposition to some adverse reactions such as increased alkaline phosphatase or hyperammonemia.
- CYP2C9-status guided refinement of valproate therapy can contribute to the avoidance of misdosing-induced side effects in children.

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### **Conflict of interest & Financial disclosure**

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. We declare that there are no personal conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Table 1. Demographic data of pediatric patients involved in the study.

Patients		Number of patients	Age (year)*	Body weight (kg)*	Boys/girls
<b>Control group</b>		47	8 (0.13 – 15)	27 (4.6 – 62)	31/16
<b>CYPtest group</b>		52	6.25 (0.5 – 15)	21 (6 – 67.5)	24/28
<i>CYP2C9*1/*1</i>	Normal expressers	12	4 (0.5 – 11)	15 (6 – 32)	4/8
	Low expressers	25	7 (0.6 – 15)	25 (7 – 67.5)	12/13
	High expressers	1	4.5	15	1/-
<i>CYP2C9*1/mut</i>	Normal expressers	9	4 (3 – 15)	19 (14.5 – 52)	5/4
	Low expressers	4	7.5 (4 -15)	22.5 (15 – 60)	1/3
<i>CYP2C9*2/*2</i>		1	7	25	1/-

\*: median (range); *CYP2C9\*1/mut*: *CYP2C9\*1/\*2* or *CYP2C9\*1/\*3*

Table 2. Patients' clinical parameters (VPA serum concentration, hematological and liver function parameters, calcium and phosphorus levels, incidence of side effects) one month after the beginning of VPA treatment.

Parameter	Control group (N=47)	CYPtest group (N=51)	Significance
VPA concentration ( $\mu\text{g/ml}$ )	85.0 $\pm$ 36.02	72.0 $\pm$ 21.05	ns
Ratio of patients out of the VPA therapeutic range	21/47	9/51	<b>P=0.004</b>
Deviation from the VPA therapeutic concentration ( $\mu\text{g/ml}$ )	21.2 $\pm$ 16.17	2.98 $\pm$ 1.90	<b>P&lt;0.001</b>
Alkaline phosphatase (unit/l)	545.1 $\pm$ 235.81	236.0 $\pm$ 138.76	<b>P&lt;0.001</b>
Ratio of patients with alkaline phosphatase exceeding the normal range	23/47	2/51	<b>P&lt;0.001</b>
Aspartate aminotransferase (unit/l)	27.3 $\pm$ 9.77	27.5 $\pm$ 8.66	ns
Alanine aminotransferase (unit/l)	16.1 $\pm$ 7.93	15.8 $\pm$ 8.50	ns
$\gamma$ -glutamyl transferase (unit/l)	15.6 $\pm$ 7.02	14.3 $\pm$ 8.51	ns
Calcium (mmol/l)	2.23 $\pm$ 0.434	2.41 $\pm$ 0.121	ns
Phosphorus (mmol/l)	1.64 $\pm$ 0.533	1.52 $\pm$ 0.297	ns
Red blood cell count ( $\times 10^{12}/\text{l}$ )	4.5 $\pm$ 0.48	4.6 $\pm$ 0.46	ns
White blood cell count ( $\times 10^9/\text{l}$ )	7.0 $\pm$ 2.19	7.9 $\pm$ 3.22	ns
Platelet count ( $\times 10^9/\text{l}$ )	227.1 $\pm$ 88.06	245.4 $\pm$ 70.81	ns

ns: not significant (P>0.05)

Table 3. Incidence of side effects one month after the beginning of VPA treatment.

Side effects	Control group (N=47)	CYPtest group (N=51)	Significance
Serious:	14/47	2/51	<b>P&lt;0.001</b>
Hyperammonemia	8/47	1/51	<b>P=0.01</b>
Nausea	1/47	1/51	ns
Thrombocytopenia	2/47	0/51	ns
Purple spots on the skin	1/47	0/51	ns
Confusion, loss of consciousness	2/47	0/51	ns
Mild:	18/47	10/51	<b>P=0.05</b>
Hair loss	3/47	2/51	ns
Somnolence	6/47	4/51	ns
Enuresis	2/47	1/51	ns
Fatigue	3/47	1/51	ns
Changes in appetite, weight gain	3/47	2/51	ns
Mood swings	1/47	0/51	ns

ns: not significant (P>0.05)

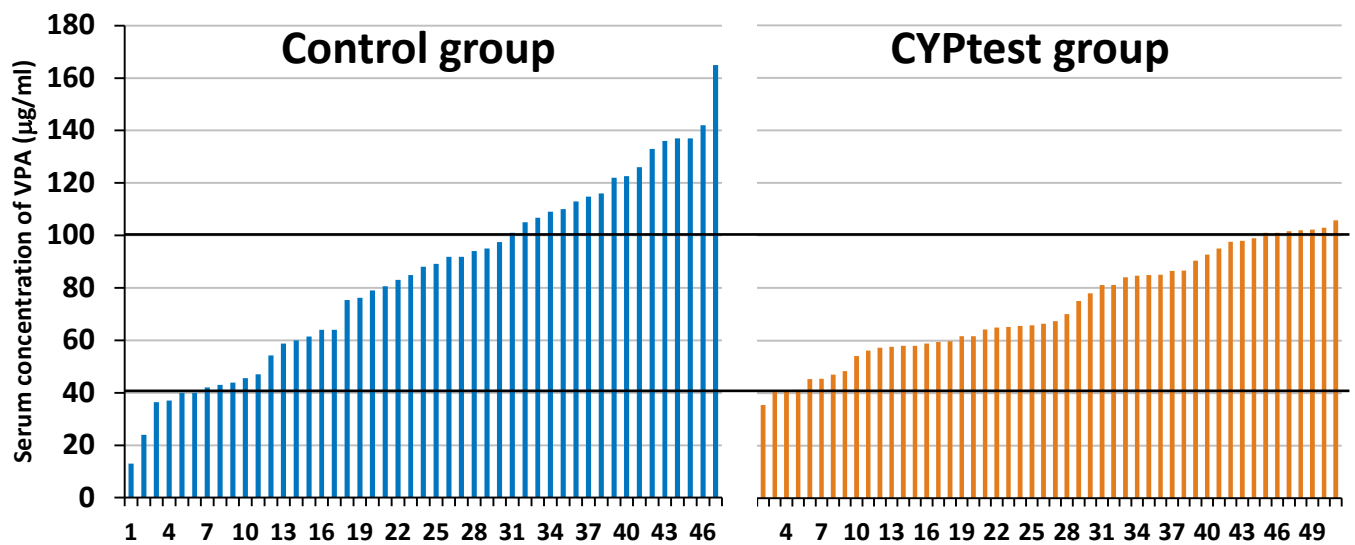


Figure 1. Serum VPA concentrations in pediatric patients one month after the beginning of VPA treatment. (Control group N=47, CYPtest group N=51) Therapeutic VPA concentration range is marked between 40 and 100 µg/ml.